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DRUG DISCOVERY

Evaluating the effects of *Mucuna urens* (Ibaba) on the body/organ weights and some haematological indices in male mice

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Histomorphological assessment of the testes and some blood parameters of mice exposed to the ethanolic seed extract of *Mucuna urens* were investigated in 24 male mice weighing between 20-30g. The mice were divided into 4 groups of 6 animals each. Group 1 was the control while group 2, 3 and 4 were the experimental groups. 80 seeds of *Mucuna urens* were used and the content was extracted using 1000mls of absolute alcohol. Filtered and dried in water-bath of 45°C. 1000mg of the extract was dissolved in 50mls of distilled water and 50mls of 30% of tween 80. The extract was administered orally for 7 days. Group 1 was administered 5ml distilled water, group 2 were administered 100mg/kg of ethanolic seed extract of *Mucuna urens*, group 3 were administered 200mg/kg of ethanol seed extract of *Mucuna urens* and group 4 were administered 300mg/kg of ethanolic seed extract of *Mucuna urens*. After sacrifice, cardiac puncture was done for hemoglobin (HB) count and Pack cell Volume (PCV) determination. Data were analysed using descriptive statistics and ANOVA. Conclusively, the ethanolic seed extract of *Mucuna urens* is anti-anaemic especially at low dose and produce no significant gain in weight in all dosages.

INTRODUCTION

This woody liana has large trifoliate leaves with entire margins and spaced secondary veins, and a swollen pulvinus [1]. It also has asymmetrical veins and leaflets. Because it is a liana, it grows spiraled around other plants in the area. The Horse Eye Bean inflorescence contains zygomorphic, pealike flowers with 10 stamens, and the greenish, cream-colored flowers are arranged in a spiral in groups of three on the stem [2]. This hanging chandelier-like cluster on the liana is found on the end of the peduncles, which can be of varying lengths up to 10 m [3]. The Leaves of *Mucuna urens* are alternate, trifoliolate and usually with leaflets which are coriaceous and glabrous. The size of the *Mucuna urens* leave is 7-14 × 4.5-7cm. The margins of the entire upper surface is dark green, shiny and glabrous and the margins of the entire lower surface is light green (purple on juvenile leaflets), shiny, glabrous, with prominent puberulent venation. The terminal leaflet is elliptical or oblong and the apex is acuminate, the base is rounded. The lateral leaflets is asymmetrical, elliptic-lanceolate, the apex acuminate, the base unequal and truncate-obtuse. The petiolules are slightly thickened, 5-6 mm long, glabrous or puberulent. The stipels are absent. The petioles are 4-9 cm long, striate and thickened at the base. The stipules are deciduous [4].

The pod of some species are covered in coarse hairs that contain the proteolytic enzyme mucunain and cause itching and blisters when it come in contact with the skin. *Mucuna* pod hairs are common ingredients in itching powder. The fruits of *Mucuna urens* are large, dehiscent brown pod which is about 15cm long. The pod has a wrinkled appearance and it is covered in an irritating golden hairs. Each pod contains around three large black seeds. The seed pod is covered with irritant hairs that are readily dislodged and can contaminate clothes or other objects. The hairs act as a mechanical-chemical skin-irritant and, even if only one of the hairs get on the skin, itch can be felt. Reddening of the skin and small papules is a common sign of the skin having contact with the pod hairs. Blindness can occur if there is physical eye contact with the pod and this can be treated by using adhesive tape and washing with water and soap, if cases are serious a doctor can be seen immediately [4].

The fruit of *Mucuna urens* is a legume, freely dehiscent and elongated, straight and ellipsoidal. Fruit exerted from calyx is internally septate between the seeds. The fruit is hairy which could be 2-seeded or 3-10 seeds. The seeds is ovoid to rounded in outline with smooth surface, it seeds may be olive, brown, or black [4]. Sap from the cut *Mucuna urens* is rubbed on sprains, rheumatic areas, contusions, sore muscles and used for children's fever. Leaf of *Mucuna urens* is used for a wash to relieve abdominal pains by cold water infusion of the crushed leaves [5]. Root of *Mucuna urens* mixed with honey is used to combat cholera. The hairs of *Mucuna urens* are mixed in molasses syrup and drunk as a vermifuge to expel intestinal worms, with a chaser of rhubarb juice. Cataplasm of the bark and ground seeds of *Mucuna urens* is recommended for inguinal hernia in French Guiana. Hairs on the fruit

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cause severe itching on the skin. Seeds of *Mucuna urens is* used for itches. Some parts of *Mucuna urens* are used for treating intestinal worms [5]. The mineral composition of *Mucuna urens* include: potassium (2000mg/100g), sodium is (26.67 mg/100g), phosphorus (87.72 mg/100g), calcium (36.06 mg/100g), magnesium (54.05 mg/100g), iron (15.91 mg/100g), copper (3.48 mg/100g) and zinc (0.03 mg/100g) [6].

MATERIALS AND METHODS

Collection of *Mucuna* Seeds

Mucuna urens seeds were obtained from a local market in Uyo Local Government Area of Akwa Ibom State. The seeds were identified and authenticated in the Department of Pharmacy, University of Uyo.

Experimental Animals

Twenty four male mice, weighing between 20g – 30g were obtained from animal house of Faculty of Basic medical sciences, university of Uyo. They were housed and acclimatized in the animal house for three weeks, under standard laboratory conditions. The mice were divided into four groups (Groups A, B, C and D) containing six mice each. They were housed in rubber cage with metallic meshed covering. The animals were feed daily throughout the course of acclimatization and administration with vital grower mash obtained from local market and clean water were provided ad libitum. The feed and water were provided in stainless plates and feeding bottles respectively and their faces were cleaned out from the cages twice weekly. They were identified by different colour markings on their tails raging from red, green and black. All the animals were handled and cared for in accordance and in compliance with applicable guidelines and standard for the care and use of laboratory animals.

Extract Preparation for Administration

The *Mucuna urens* seeds were crushed with grinding stone to remove the epicarp and the mesocarp was removed with knife leaving the endocarp. These were grinded with grinding machine to obtain fine powdered particles. The particles were soaked in absolute ethanol of 800 mls for 72 hrs. The crude extract was separated from the suspended particles by filtration. The filtrate was concentrated in water bath of 45°C for 48hrs and stored in refrigerator at 4°C. The sticky concentrated ethanol free *Mucuna urens* seed extract (1000mg) were mixed with 50ml of water and 50ml of 30% tween 80. Thus 100ml of 15% tween 80 was used to dissolve the extract. The extract was administered orally with the aid of feeding tube attached to 10ml syringe. The administration was done in accordance to the body weight of the mice.

Experimental Design

The animals which were divided into four different groups received dosages as follows:

- Group 1 (Control group) was administered with 5ml distilled water.
- Group 2 (Low dose) administered with 100mg/mlof the extract solution.
- Group 3 (Medium dose) administered with 200mg/ml of the extract solution.
- Group 4 (High dose) administered with 300mg/ml of the extract solution.
- Mucuna seed extract was given to the animals for one week.

Body/Organ Weight Determination

The Body/Organ Weights of the animals were determined using an electronic weighing balance.

Determination of Haematological Parameters

The blood samples were obtained through cardiac puncture of the mice for the determination of the some haematological parameters: Packed Cell Volume (PCV) and haemoglobin (Hb) count, using the Capillary Tube Method and Spectrophotometric Method respectively [7]. The blood samples were collected in EDTA sample bottles for haematological assay. This was then centrifuge at 33.5g for 15 minutes using a Uniscope Laboratory Centrifuge.

Animal sacrifice

After one week of administration the animal were weighed again and sacrificed using chloroform vapor. The animals were placed in a glass jar and chloroform vapour was oozed into it. It was covered with thick place glass for 5minutes. After anesthesia, the animals were placed on a rectangular wooden surface that was sterilized. The testis were surgically removed, immediately the weight of the testis of individual animals in different groups were determined after sacrifice by placing the harvested tissues on the electronic weighing balance and viewed to record the displayed weight on the screen.

Statistical Analysis

Data were analyzed using mean, standard deviation, summation and ANOVA and were represented on histogram.

RESULTS & DISCUSSION

The result of group 1 mice administered with distilled water has the final weight less than every other group but higher than the initial body weight. Group 2, 3 and 4 which were administered 100mg/kg, 200mg/kg and 300mg/kg of ethanolic extract of *Mucuna urens* respectively were higher than the control group. This increase were dose dependent, but there were decline in weight of group 3 and 4 administered 200mg/kg and 300mg/kg of ethanolic extract of *Mucuna urens* respectively. This decline is in total disagreement with the research conducted by Udoh and Ekpenyong, 2001 [8] on the effect of *Mucuna urens* on gunea-pig at high dose of 140mg/kg which concluded that there were no weight loss.

The result of control group of mice administered with distilled water has the weight of testes less than every other group, this increase in weights were dose dependent. This increase in weight of group 2, 3, and 4 administered 100mg/kg, 200mg/kg and 300mg/kg of ethanolic extract of *Mucuna urens* respectively, could be due to oedema.

The blood analysis of group 1 in fig. 3 showed that packed cell volume of this control group is not within the range of 40% - 45% rather is 30%. This is the clinical range for human packed cell volume according to Sembulingam and sembulingam, 2012 [9], though the control group packed cell volume is not within the normal range does not make it abnormal since feed and water were given at ad libitum and the mice were administered distilled water. On account of this investigation, the control group will be the basis of comparison with the experimental group.

In group 2 mice administered 100mg/kg of ethanolic extract of *Mucuna urens* showed a packed cell volume of 40% which is within the clinical range of 40% - 45% according to Sembulingam and Sembulingam, 2012 [9]. This may be due to increase in haematopoietic activity caused by some minerals in *Mucuna urens* which enhances haemoglobin formation; these include copper and iron according to

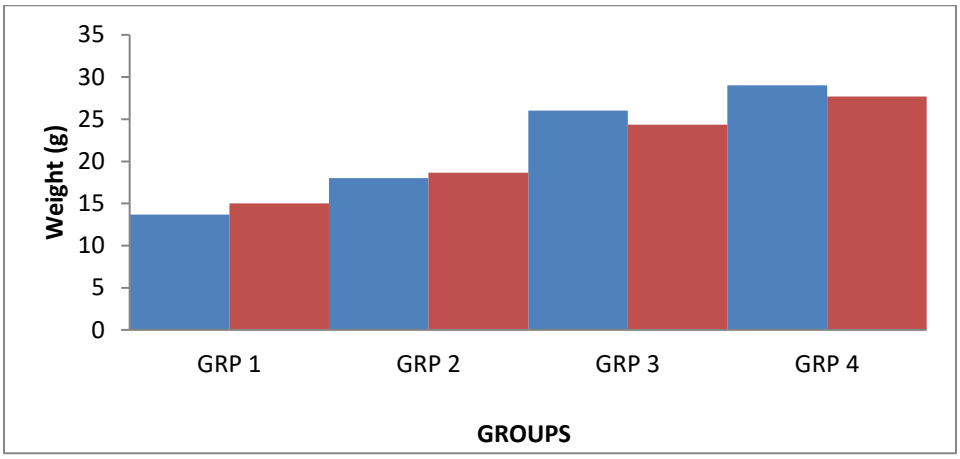

Figure 1 Histogram representing an initial weight of mice against final weight of Mice. It shows the changes that occur in the weight of mice during the course of administration

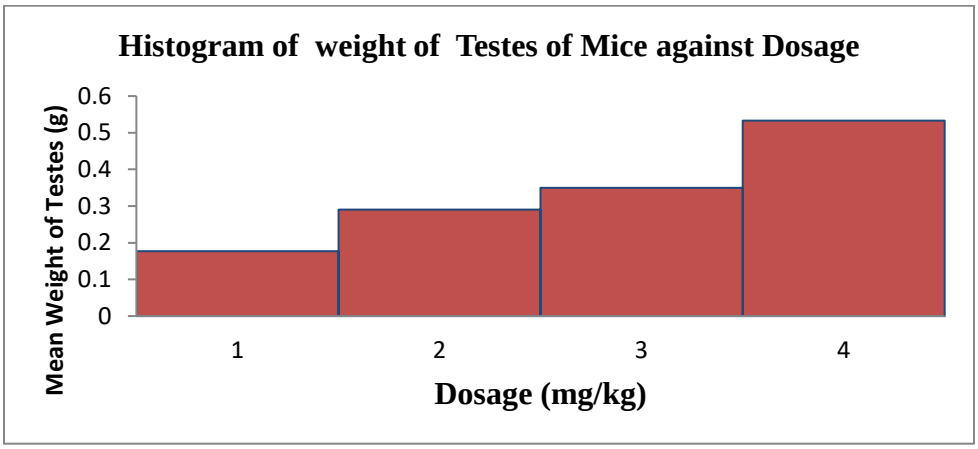

Figure 2 Histogram representing weight of Testes of Mice

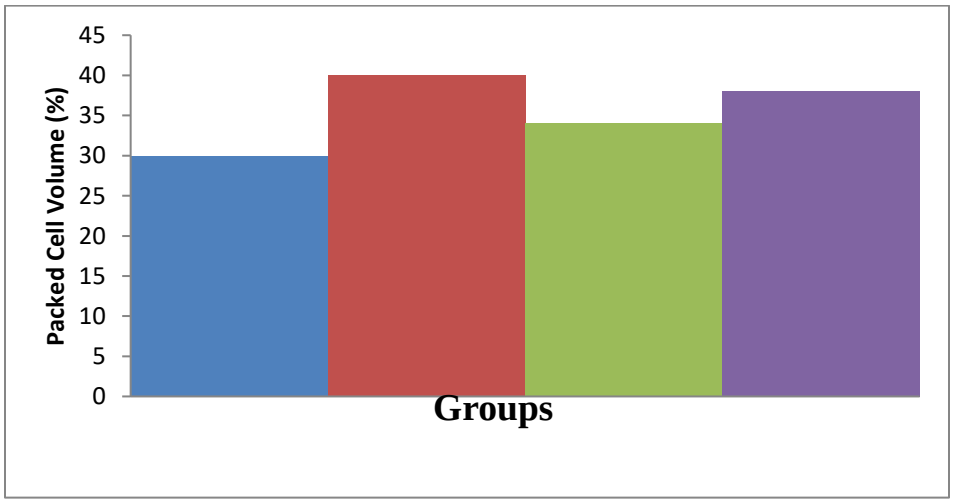

Figure 3 Histogram representing packed cell volume (PCV) of blood in percentage in different groups of mice. The average of each group was used

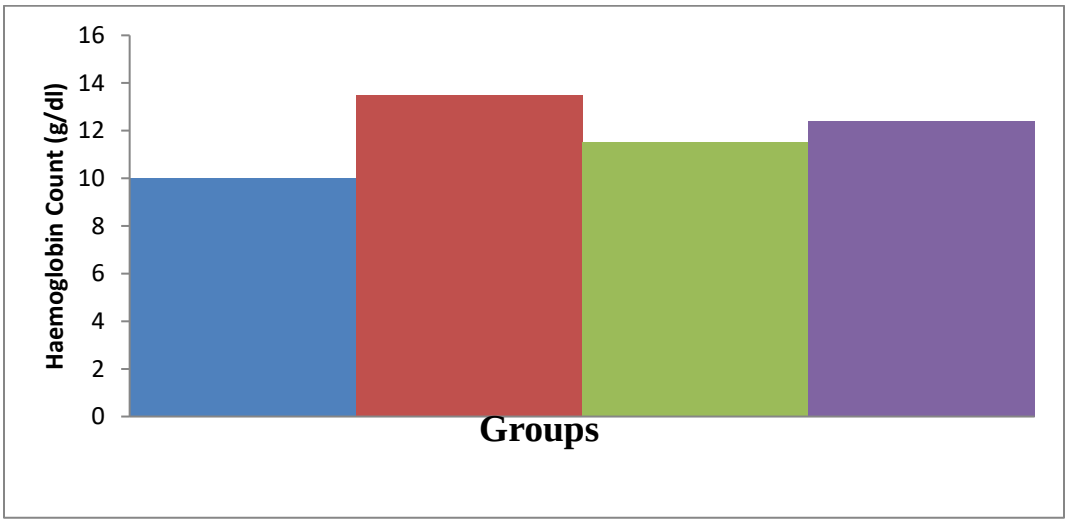

Figure 4 Histogram representing Hemoglobin of blood in Haemoglobin Count (g/dl) in different groups of mice

Umoren, Effiong and Akpan, 2007 [6], it also contain ascorbic acid an erytropoietic factor, according to Chikwendu, Ayogu and Nwaezza, 2014 [10], these together enhances increase the high percentage of Packed Cell Volume since according to Sembulingam and sembulingam, 2012[9], polycythemia contribute to high Packed Cell Volume.

In group 3 mice administered 200mg/kg of ethanolic extract of *Mucuna urens*, showed a packed cell volume of 38% which is within the clinical range of 40% - 45% according to Sembulingam and Sembulingam, 2012[9]. The increase in packed cell volume above the control group may be due to the anti-anaemic effect of *Mucuna urens* leading to polycythemia but it decrease by 2% compared to the low dose may be due to the presence of phatate according to Pâivi, Ekholm and Virkki *et al.*, 2003[11] which has a strong binding affinity for some minerals in *Macuna urens* which enhances haemoglobin formation, these are copper and iron. This implies that copper and iron will not be available for haemoglobin maturation leading to decrease available matured haemoglobin.

In group 4 mice administered 300mg/kg of ethanolic extract of *Macuna urens*, showed a packed cell volume of 38% which is within the clinical range of 40% - 45% according to Sembulingam and Sembulingam, 2012[9]. The increase in packed cell volume above the control group may be due to the anti-anaemic effect of *Mucuna urens* leading to polycythemia but it decreased by 6% compared to the low dose. This may be due to the presence of phatate according to Pâivi, Ekholm and Virkki *et al.*, 2003[11] which has a strong binding affinity for some minerals in *Mucuna urens* which enhances haemoglobin formation, these include copper and iron. This implies that copper and iron will not be available for haemoglobin maturation leading to decreased available matured haemoglobin.

Haemoglobin count has its normal clinical range as 13-18g/dl for a normal African man. This clinical range is according to Udombon, 2013. The haemoglobin count for the control group is 10g/dl. This result is not within the clinical range of Udombon, 2013[7] yet this does not make it clinically abnormal since it is for mice given feed and water ad libitum.

The blood analysis of the haemoglobin count of group 2 administered 100mg/kg of ethanolic extract of *Mucuna urens*is 13.50g/dl. This result is within normal clinical range of 13-18g/dl according to Udombon, 2013. This result is above the haemoglobin count for control group and this may be due to the ascorbic acid, riboflavin, zinc and iron chemical component of *Mucuna urens* according to Umoren, Effiong and Akpan, 2007[6] which contribute to maturation of Haemoglobin. This implies that there is an increase in haemoglobin count at low dose since this maturation factor will enhance quick haemoglobin maturation. The haemoglobin count of group 3 mice administered 200mg/kg of ethanolic extract of *Mucuna urens* is 11.50g/dl. This result is not within normal clinical range of 13-18g/dl according to Udombon, 2013[7] but it is above the value of the control group. This increase in the haemoglobin count of group 3 is because of the presence of ascorbic acid and folic acid which are erythropoietic factors present in *Mucuna urens* according to Chikwendu, Ayogu and Nwaezza, 2014[10]. Despite the presence of these erythropoietic factors and higher dose of ethanolic extract of *Mucuna urens*, the value is not as high as that of low dose because of anti-nutrient factor that inhibits absorption.

The haemoglobin count of group 4 mice administered 300mg/kg of ethanolic extract of *Mucuna urens* is 12.40/dl. This result is not within normal clinical range of 13-18g/dl according to Udombon, 2013[7] but it is above the value of the control group. This increase in haemoglobin count may be due to the presence of ascorbic acid, zinc and copper.

CONCLUSION

The result obtained from this research shows that *Mucuna urens* is anti-anaemic at low dose and does not show forth significant gain in weight in all the doses.

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